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# Effect of Temperature and Drug Therapy on Anthrax Intoxication. (31823)

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Stress of the test animal with low ambient temperature changes the toxicity of bacterial toxins(1,2). Cold temperature has also been known to influence the action of certain drugs in warm blooded animals(3,4,5,6,7). Recently, Stahnke(2) interacted these two independent variables (temperature and drug) with rattlesnake venom and scorpion venom and observed that both venoms increased in toxicity in the rat under cold stress. Rats receiving the drug epinephrine reacted similarly. In a similar study with *S. marcescens* endotoxin, mice were made more susceptible to the lethal effects of the toxin following cold exposure. Previte and Berry(1) showed that cortical hormones effectively counteracted the toxicity of the toxin at both room and low temperatures. Both studies attributed these apparent changes in toxicity to the physiological effects of stress rather than the temperature *per se*.

In this paper we report initial experiments which suggest that temperature and certain drugs alter the susceptibility of the rat to anthrax toxin. The results indicate that reactions were the opposite to other reported bacterial toxins and snake venoms and suggest alterations other than physiological stress.

The studies were based on the proven homogeneous susceptibility of the Fischer 344 rat to anthrax toxin(8) and the precise assay procedure for anthrax toxin developed utilizing this animal(9). Anthrax toxin was pre-

pared by the method of Haines, Klein, and Lincoln(9) and concentrated by drying. An initial dilution in gel phosphate to 400 units/ml was made and further diluted in saline as needed so that intravenous challenge dose was contained in 1 ml.

For the studies in which rats were stressed by being held at controlled temperatures, the animals were challenged with toxin and placed in environments of 4 ± 1°C, room temperature, (24° ± 2°C) and 38° ± 1°C. Body temperature was determined by a 1.0-inch, 23-gauge insulated junction thermocouple needle, Model HTI-AYP-1.00-CC-2,* inserted 1 to 1.5 cm into the rear leg muscle. Temperature was read directly from a Model 153 × ( '4-X-81, 4 probe temperature recorder† calibrated against a Bureau of Standards mercury thermometer. Further treatments consisted of pharmalogical doses of steroids (adrenocorticotropic hormone ACTH—10 units per rat; hydrocortisone-10 mg per rat) and pharmalogicals (caffeine 0.005 mg per rat; N-allyl-morphine 0.1 mg per rat) arranged in factorial or block design to determine the interactions between 4° *vs* 24°C temperature and/or intravenous injection of barbituate (Nembutal 0.2 ml IV followed by 0.2 ml in testicle). The effect of antiserum on response to anthrax toxin at 4° and 24°C was tested in factorial arrangements. Each ml of antiserum neutralized *in vitro* about 7100 rat units of toxin. Treatments studied included 3 antiserum levels each administered intravenously to the test animals at predetermined time after challenge with toxin.

Body temperatures were measured in 6 groups of rats (Fig. 1), 3 control groups and 3 challenged with lethal doses of anthrax toxin. One each of the challenged and the control groups was maintained at 37°, 24°, and 4°C.

Body temperature of rats held in an environment of 37°C was increased over that of animals held at 24°C, approximately 2.5°C during the first 10 minutes, peaked 4°C above normal and then slowly adjusted to a fairly stable temperature of 3.0°C above normal. Toxin challenged rats at this temperature increased their body temperature approximately 0.5°C over unchallenged animals held at the same temperature and at death did not fall below that of the control. The mean time to death (MTD) was 36 minutes shorter than animals held at 24°C.

Throughout the entire 200-minute observation period the unchallenged rats at 24°C temperature had a body temperature of 38° ± 1°C. The body temperature of rats challenged with 27 units of toxin was maintained for the first 70 minutes, then declined to about 36°C at the MTD of 121 minutes.

At 4°C, body temperature of unchallenged rats was 1-2°C lower than controls at room temperature. Toxin challenged rats maintained their temperature for the first 50 minutes. Body temperature then rapidly declined to 31°C at the time the first animal died, to 26°C at the MTD of 149 minutes and 24°C at the time of death of the last animal. Hypothermia is clearly evident after toxin challenge in animals maintained at 24°C, and it is more severe in animals housed at 4°C. These data show anthrax toxin in not pyrogenic and closely follows the results reported on temperature infection relationship of the disease in the spore challenged guinea pig(10,11).

Further tests at 4° and 24°C with rats given 8, 16, 24, or 32 units of toxin showed that cold stressed rats died with significantly ($P > 0.01$) extended MTD, and in both groups the MTD was related to the toxin dosage. The 8 unit dose of toxin was nonlethal at 24°C; however, all animals at 4°C died. At 37°C all animals died and MTD was shorter than at 24° and 4°C.

In spite of the increased susceptibility to toxin by stress at 4°C, it was possible that the increased MTD might allow a longer time period during which treatment with antiserum would prevent toxic death. This concept was tested in an experiment in which 3 dosages of toxin, 15, 30, and 60 units, were administered to rats held at 4°C. Treatment with 0.5, 1.0, or 3.0 ml of antiserum was initiated at 30, 45, 60, or 90 minutes after toxin challenge. Results are given in Table I. At

* High Temperature Instrument Co., Philadelphia, Pa.

† Minneapolis Honeywell REG Co., Brown Instrument Division, Philadelphia, Pa.

toxin dosages of 30 and 60 units, no treatment resulted in survivors; however, at 15 units, animals survived if antiserum were given at or prior to 60 minutes, but died when administration of antiserum was delayed until 90 minutes. Although stress at 4°C again extended time to death, there was no statistically significant interaction with antiserum.

A test of selected chemicals used in pharmalogic dosages and interactions among the

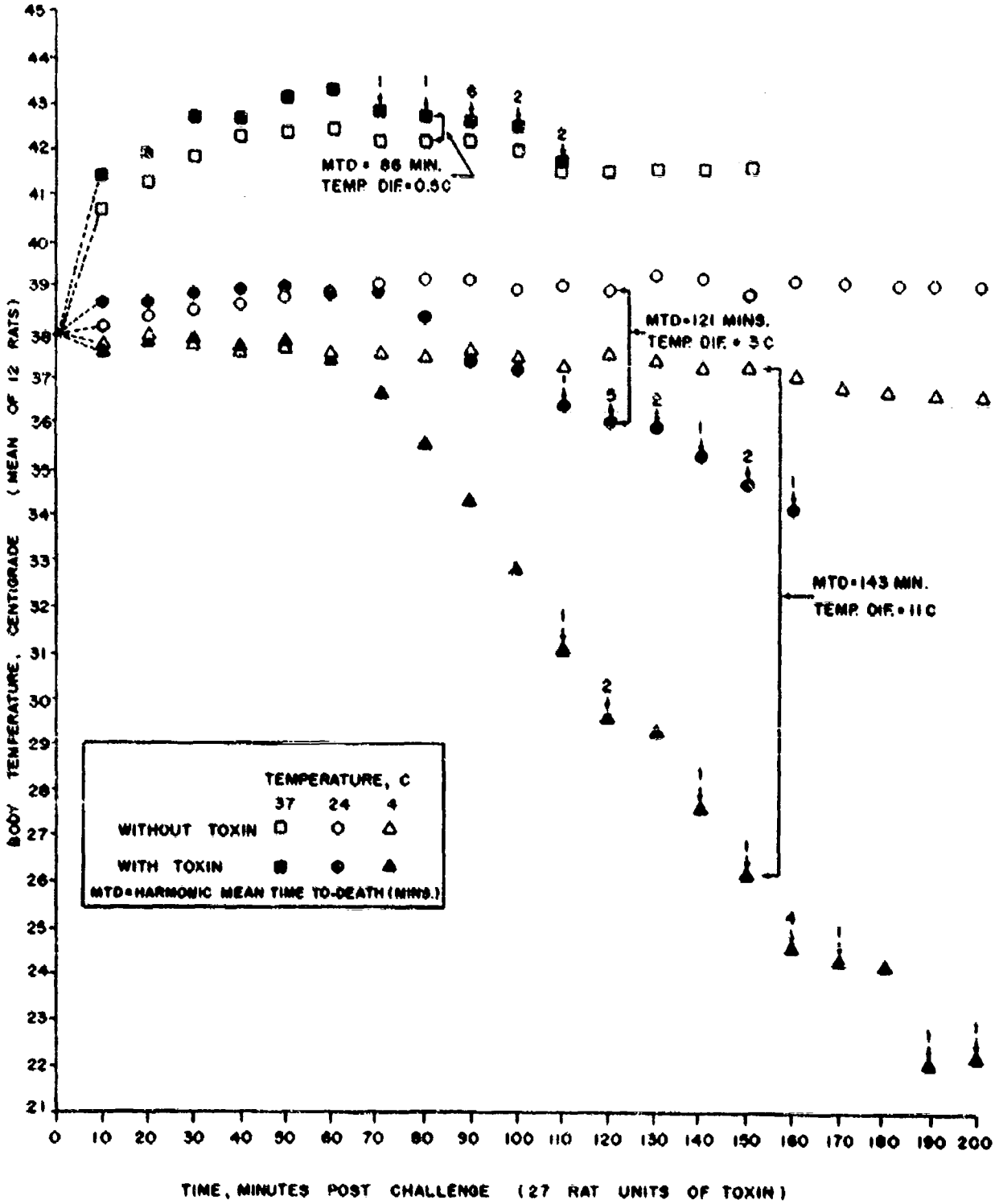


FIG. 1. Time temperature relationship of rats dying with a lethal dose of anthrax toxin (30 units/rat). Numbers 1 through 6 indicate number of animals dying at indicated time ± 5 min. MTD, mean time to death.

TABLE I. Survival Time of Rats Challenged with Anthrax Toxin and Treated with Antiserum.

| Temp (C) | Dosage (ml) | Time administered after toxin (min) | Harmonic mean time to death (min)* for challenge with indicated units of toxin | | |
|---|---|---|---|---|---|
| | | | 15 | 30 | 60 |
| 22 ± 2 | None (control) † | | 233 | 100 | 80 |
| | .5 | 30 | S‡ | 217 | 72 |
| | | 45 | S | 122 | 89 |
| | | 60 | S | 101 | 72 |
| | | 90 | 322 | 96 | 84 |
| | 1.0 | 30 | S | 38 | 72 |
| | | 45 | S | 128 | 71 |
| | | 60 | S | 108 | 68 |
| | | 90 | 238 | 103 | 73 |
| | 3.0 | 30 | S | 340 | 88 |
| | | 45 | S | 139 | 79 |
| | | 60 | S | 126 | 75 |
| | | 90 | 269 | 109 | 83 |
| 4 ± 1 | None (control) | | 429 | 121 | 99 |
| | .5 | 30 | S | 869 | 93 |
| | | 45 | S | 138 | 83 |
| | | 60 | S | 134 | 83 |
| | | 90 | 501 | 81 | 96 |
| | 1.0 | 30 | S | 346 | 124 |
| | | 45 | S | 129 | 75 |
| | | 60 | S | 150 | 79 |
| | | 90 | 398 | 148 | 107 |
| | 3.0 | 30 | S | 609 | 224 |
| | | 45 | S | 199 | 83 |
| | | 60 | S | 164 | 85 |
| | | 90 | 573 | 188 | 75 |

* Mean of 6 rats. † Control is mean of 18 rats. ‡ Survival.

drugs and with 4° *vs* 24°C did not result in survival of animals challenged with approximately 30 units of toxin (Table II). Survival time, however, was influenced with barbituates known to depress metabolism, extending the MTD at 24°C(12) but shortened it at 4°C. The steroids, which are usually considered as minimizing the effects of stress, were without effect. Caffeine and D-allyl-morphine also were without effect. The interaction of barbituates with hydrocortisone greatly extended the MTD at 24°C but shortened it a 4°C. Other interactions were too small to be considered of practical significance.

Our work has been directed toward finding ways to extend the time during which anthrax might be successfully treated. For the progressing septicemic disease, antiserum and a bacteriostatic antibiotic rather than a bacteriocidal antibiotic used together results in maximum survival(13). This work considering only the toxemic phase of anthrax shows that the effective time for action of antiserum is limited for it is evident that toxin is irreversibly fixed to essential sites soon after injection. In this study only with 15 units of toxin could death be prevented by administration of antiserum.

Anthrax toxin is a complex toxin composed of at least 3 components (see review 14) and at least 2 components, lethal factor (LF) and protective antigen (PA), are required for lethality. It seems probable that antiserum inactivates the LF component of the toxic complex since this component disappears from the blood stream more rapidly than the LF component(15). If so, then treatment of anthrax should consider more specifically the LF component, and antigens used to produce immunity or antiserum should contain the LF component(16).

Although the cold stress (4°C) of animals challenged with anthrax toxin extended the MTD, the host increased in susceptibility as

TABLE II. Mean Time to Death Following Anthrax Toxin Challenge as Influenced by Indicated Treatments.

| Toxin (units/rat) | Treatments | Harmonic mean times to death (min)* Temperature 24°C (room) | 4°C | Difference |
|---|---|---|---|---|
| 27 ± 1 | Barbiturate (B) | 190 | 142 | —40 |
| | Hydrocortisone (H) | 116 | 139 | +23 |
| | Adrenocorticotropic hormone (A) | 123 | 160 | +37 |
| | B + H | 221 | 131 | —90 |
| | B + A | 137 | 128 | — 9 |
| | Controls | 120 | 178 | +58 |
| 30 ± 1 | Caffeine (C) | 98 | 113 | +15 |
| | D-allyl-morphine (M) | 105 | 121 | +16 |
| | Barbiturate (B) | 137 | 125 | —12 |
| | B + C | 126 | 127 | + 1 |
| | B + M | 120 | 133 | +13 |
| | Controls | 108 | 117 | + 9 |

* Mean of 6 rats.

8 units of toxin killed the 4°C stressed animals but not those held at 24°C. In that the time to death is greatly extended by 4°C stress, anthrax toxin differs from spider and snake venoms(2) and endotoxin(1); however, both anthrax toxin and endotoxin result in a hypothermic condition. The greatly increased time to death did not increase the time during which chemotherapeutic treatment against the effect of toxins might be made and prevent death. Also, none of the drugs tested at pharmacologically active levels extended the time to death enough to appear very promising as a chemotherapeutic way of preventing toxemic death after treatment with antibiotics and/or by simultaneously inactivating the toxin with antiserum and supporting the physiology of the host with drugs while detoxification of fixed toxin proceeds to a sublethal level.

*Summary.* In contrast to other known bacterial toxins and venoms, challenge with anthrax toxin resulted in a hypothermia which is as great as 14-15°C in animals stressed by holding at 4°C. In comparison with animals held at room temperature following challenge, mean time to death is shortened in those animals held at 37°C and extended at 4°C became more susceptible to anthrax toxin, being killed by 8 rat units of toxin, whereas 16 units were required to kill animals held at room temperature. Antiserum, if administered through 60 minutes, prevented death of rats challenged with 15 units of toxin and tended to extend time to death of rats challenged with higher dosages of toxin. For animals challenged with 30 units of toxin and held at 24°C, the drugs, caffeine, N-allyl-morphine, ACTH, hydrocortisone and barbituate were ineffective except for barbituate which extended time to death. All drugs were ineffective for rats stressed at 4°C.

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